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INTRODUCTION

A combinatorial approach of gene silencing and expression profiling in deciphering the roles of prion and auxiliary molecules in aberrant prion replication

Infectious, self-propagating protein aggregates (prions) and deposition of amyloid fibrils are universal components of prion-based diseases. Prion null mice are fertile, neurologically normal and show resistant to scrapie infection than their wild-type counterparts, suggesting that disease progression may be rate limited by suppressing prion expression. The underlying hypothesis is to use gene expression studies to decipher the roles of prion and known auxiliary macromolecules in aberrant prion replication and identify new targets. DNA-encoded hairpin RNAs will be used to nullify the expression of these known and newly identified targets in order to mitigate the aberrant prion replication.

BODY

Scrapie infected neuroblastoma cells were cultured following standard methods. Genomic DNA was isolated from neuroblastoma cells and prion specific gene was amplified using polymerase chain reaction. Amplified prion gene was sequenced and sequence was verified with existing sequence data base to make sure that constructed RNAi vector can encode complementary double stranded RNA molecule. DNA-encoded hairpin RNAs were constructed against the sequenced prion gene. The effectiveness of RNA interference (RNAi) was demonstrated by ability of anti-PrP construct expressing fluorescent protein as an indicator of cell specific transformation and hairpin substrate for production of small inhibitory RNAs to suppress expression of PrP as detected by immunofluorescence.

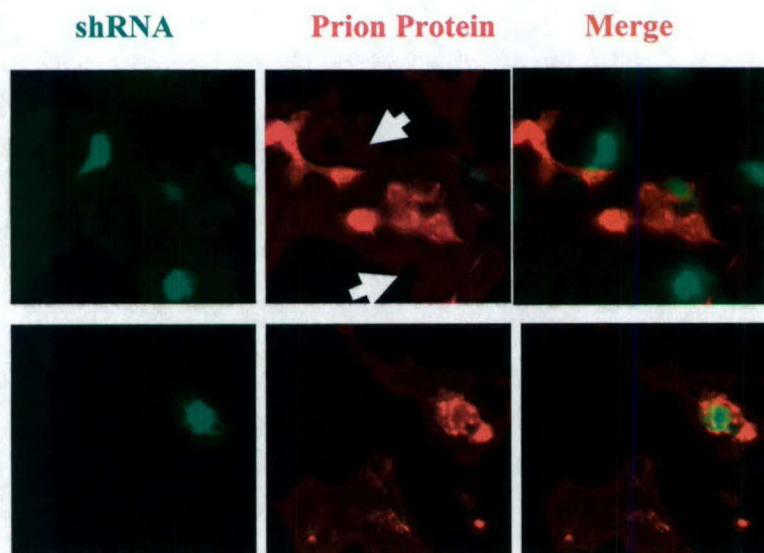


Figure Description: Upper panel shows RNAi mediated effective suppression of PrP expression (arrow marked) with reference to lower panel (control). Green color indicates certain neuroblastoma cells expressing RNAi construct, whereas Red color shows level of PrP protein expression.

KEY RESEARCH ACCOMPLISHMENTS

- Effective DNA encoded short hairpin RNA (shRNAs) constructs were generated against Prion specific gene.
- Effect of RNAi mediated suppression of prion protein expression was achieved.

REPORTABLE OUTCOMES

DNA encoded short hairpin RNAs (shRNAs) can suppress prion protein expression.

CONCLUSIONS

RNAi mediated suppression of prion protein expression in cell culture system allows us to apply similar strategy in mouse model of incipient prion disease.

REFERENCES

Rajeev Kumar, Inyoul Lee, Anna Gibson, Leroy E. Hood, George A. Carlson (2004) Use of Expression arrays to prioritize and RNAi to test prion incubation time modifier gene candidates. *Neurobiology of Aging* 25 (S2) S456-457.

APPENDICES

None